

Supporting Information

Peptoid-Peptide Hybrid Ligands Targeting the Polo Box Domain of Polo-Like Kinase 1

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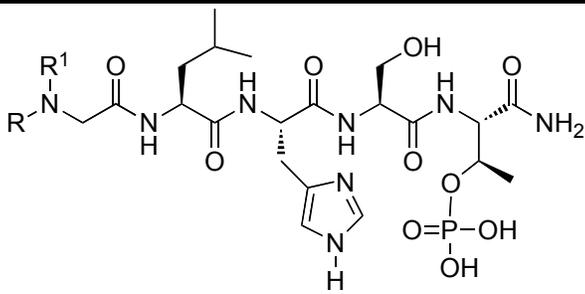
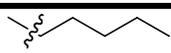
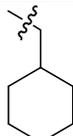
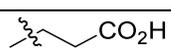
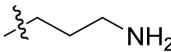
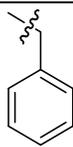
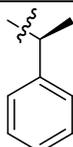
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Table S1. Low Resolution ESI-Mass Spectral Data and HPLC Purity of 6a – 6r.

					
Compound Number	R	R ₁	Expected (M + H) ⁺	Observed (M + H) ⁺	HPLC Purity
6a		Acetyl	705.3	705.8	>99%
6b		H	661.3	661.5	64%
6c		Acetyl	731.3	731.7	97%
6d		Acetyl	707.3	707.4	65%
6e		Acetyl	692.3	690.8	>99%
6f		Acetyl	725.3	725.4	79%
6g		H	697.3	697.6	79%

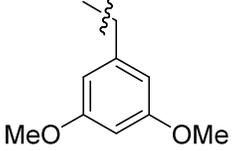
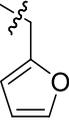
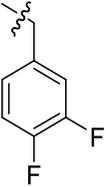
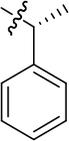
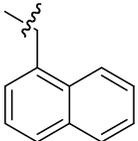
6h		Acetyl	785.3	785.9	96%
6i		Acetyl	715.3	715.9	77%
6j		Acetyl	761.3	761.8	89%
6k		H	697.3	698.0	93%
6l		H	733.3	732.6	>99%
6m	-(CH ₂) ₂ -Ph	Acetyl	739.3	739.2	>99%
6n	-(CH ₂) ₃ -Ph	Acetyl	753.3	753.3	>99%
6o	-(CH ₂) ₄ -Ph	Acetyl	767.3	767.2	>99%
6p	-(CH ₂) ₅ -Ph	Acetyl	781.4	781.3	96%
6q	-(CH ₂) ₆ -Ph	Acetyl	795.4	795.3	>99%
6r	-(CH ₂) ₇ -Ph	Acetyl	809.4	809.4	>99%

Table S2. Low Resolution ESI-Mass Spectral Data and HPLC Purity.

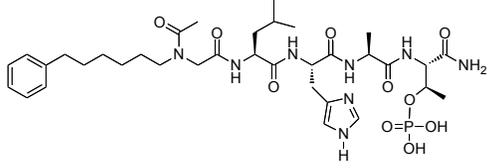
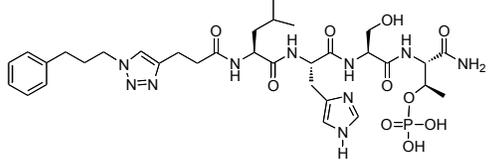
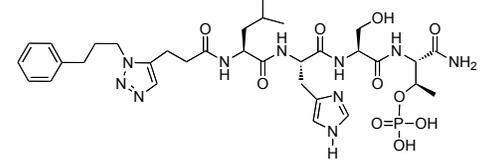
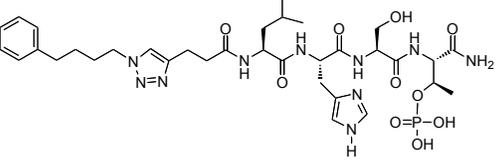
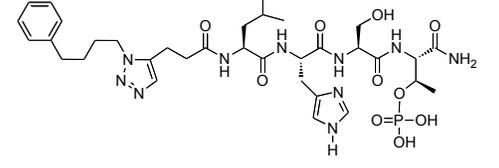
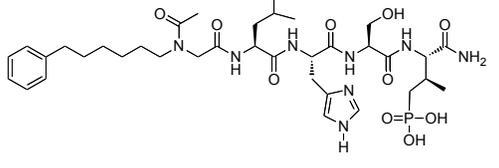
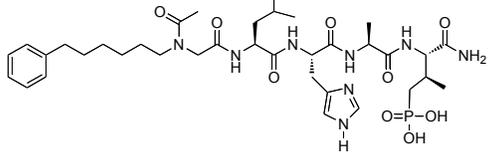
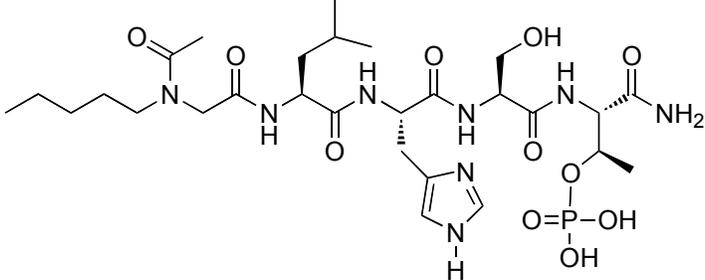
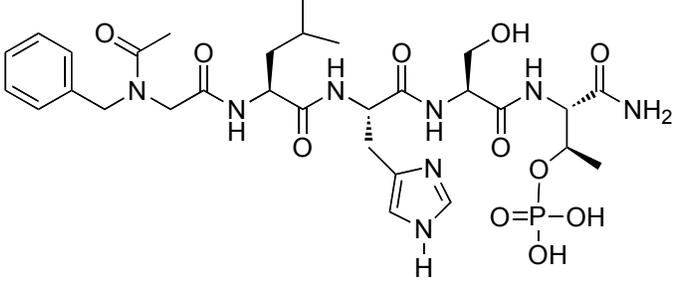
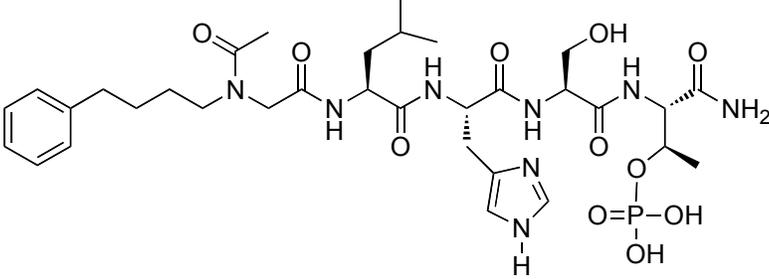
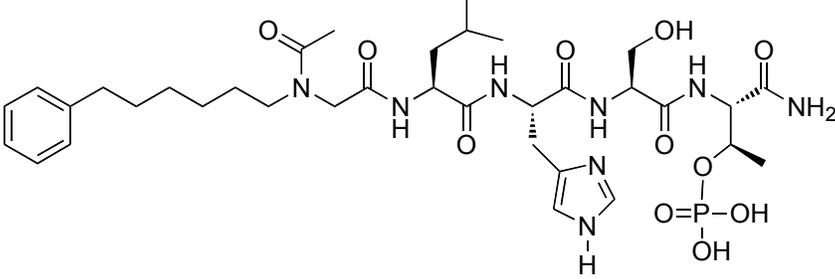
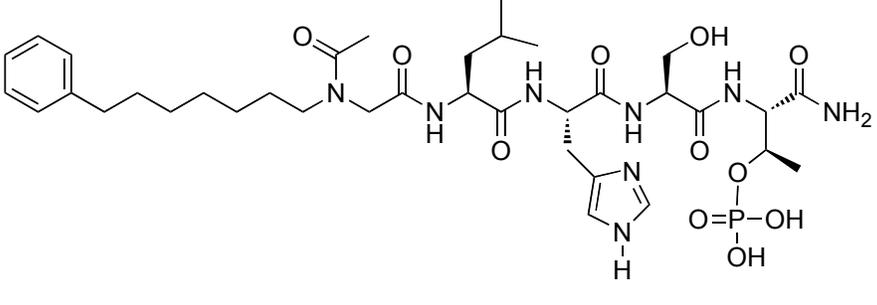
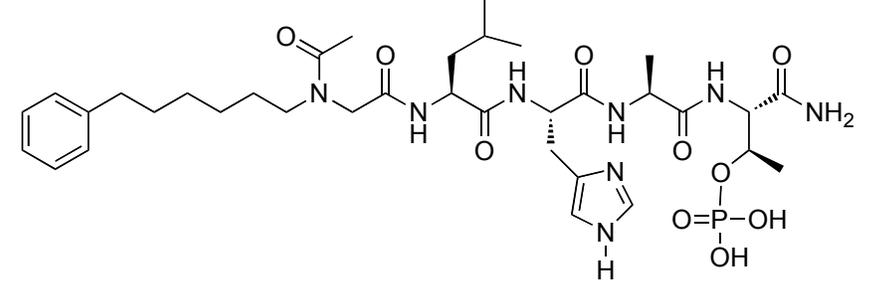
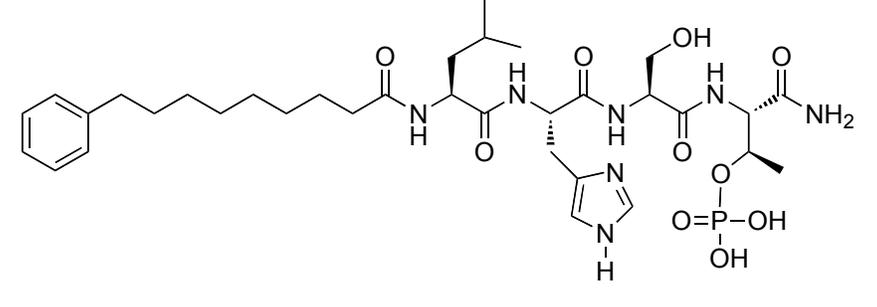
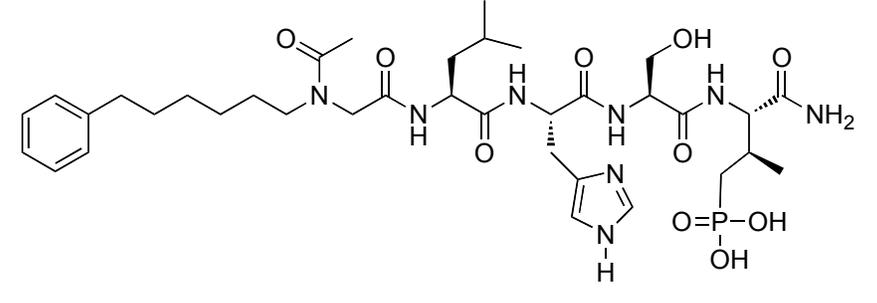
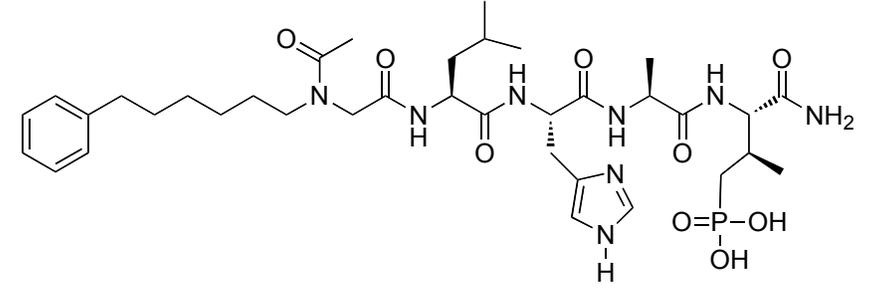
No.	Structure	Expected (M + H) ⁺	Observed (M + H) ⁺	Expected (M - H) ⁻	Observed (M - H) ⁻	HPLC Purity
6q(S4A)		779.4	779.4	777.4	777.4	100%
11a		777.3	777.3			100%
11b		777.3	777.4	775.3	775.4	100%
12a		791.4	791.2			100%
12b		791.4	791.2			100%
13		793.4	793.5	791.4	791.4	74%
13(S4A)		777.4	777.4	775.4	775.4	80%

Table S3. High Resolution ESI-MS of Selected Peptoid-peptide Hybrids.

No.	Structure	Calculated	Observed
6a		$(M - H)^-$ $C_{28}H_{48}N_8O_{11}P$ 703.3186	703.3166
6f		$(M - H)^-$ $C_{30}H_{44}N_8O_{11}P$ 723.2873	723.2846
6n		$(M - H)^-$ $C_{33}H_{50}N_8O_{11}P$ 765.3342	765.3328
6q		$(M + H)^+$ $C_{35}H_{56}N_8O_{11}P$ 795.3801	795.3783

6r		(M - H)- C ₃₆ H ₅₆ N ₈ O ₁₁ P 807.3812	807.3799
6q(S4A)		(M + H)+ C ₃₅ H ₅₆ N ₈ O ₁₀ P 779.3851	779.3846
7		(M - H)- C ₃₄ H ₅₃ N ₇ O ₁₀ P 750.3597	750.3583
13		(M + H)+ C ₃₆ H ₅₈ N ₈ O ₁₀ P 793.4008	793.4021
13(S4A)		(M + H)+ C ₃₆ H ₅₈ N ₈ O ₉ P 777.4059	777.4079

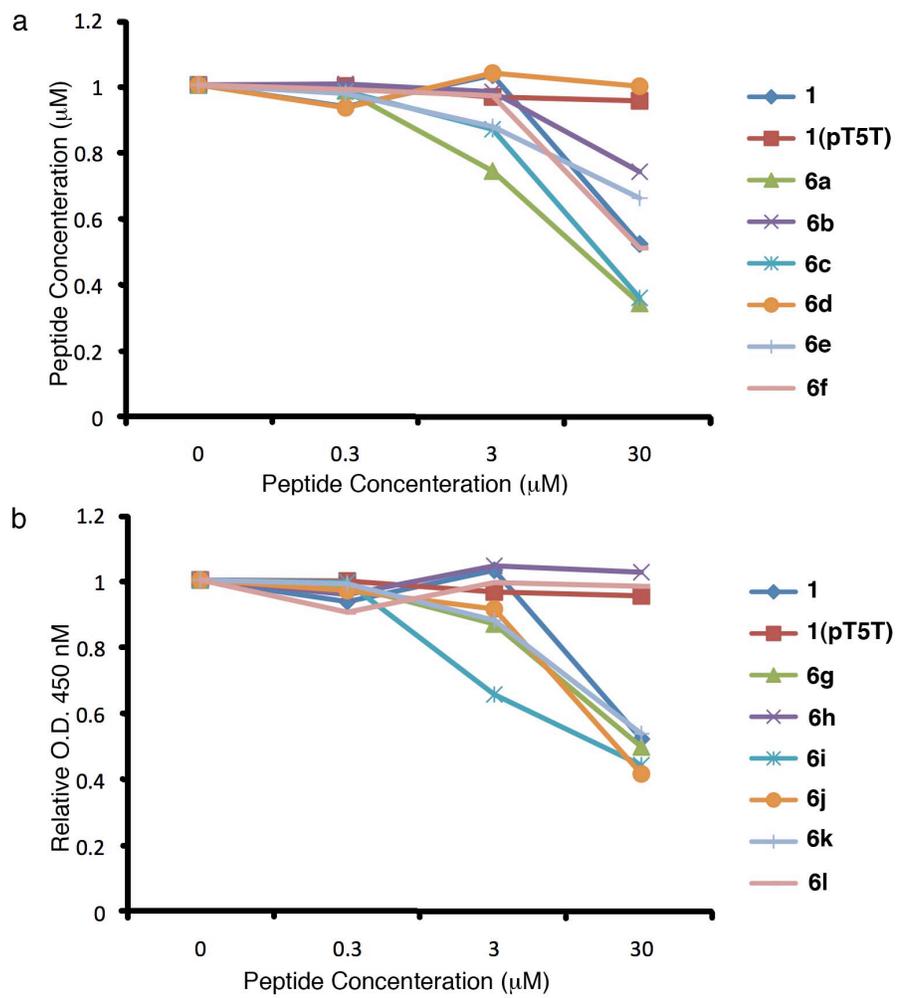


Figure S1. ELISA Plk1 PBD-binding data. Representative graphs from three independent experiments are shown.

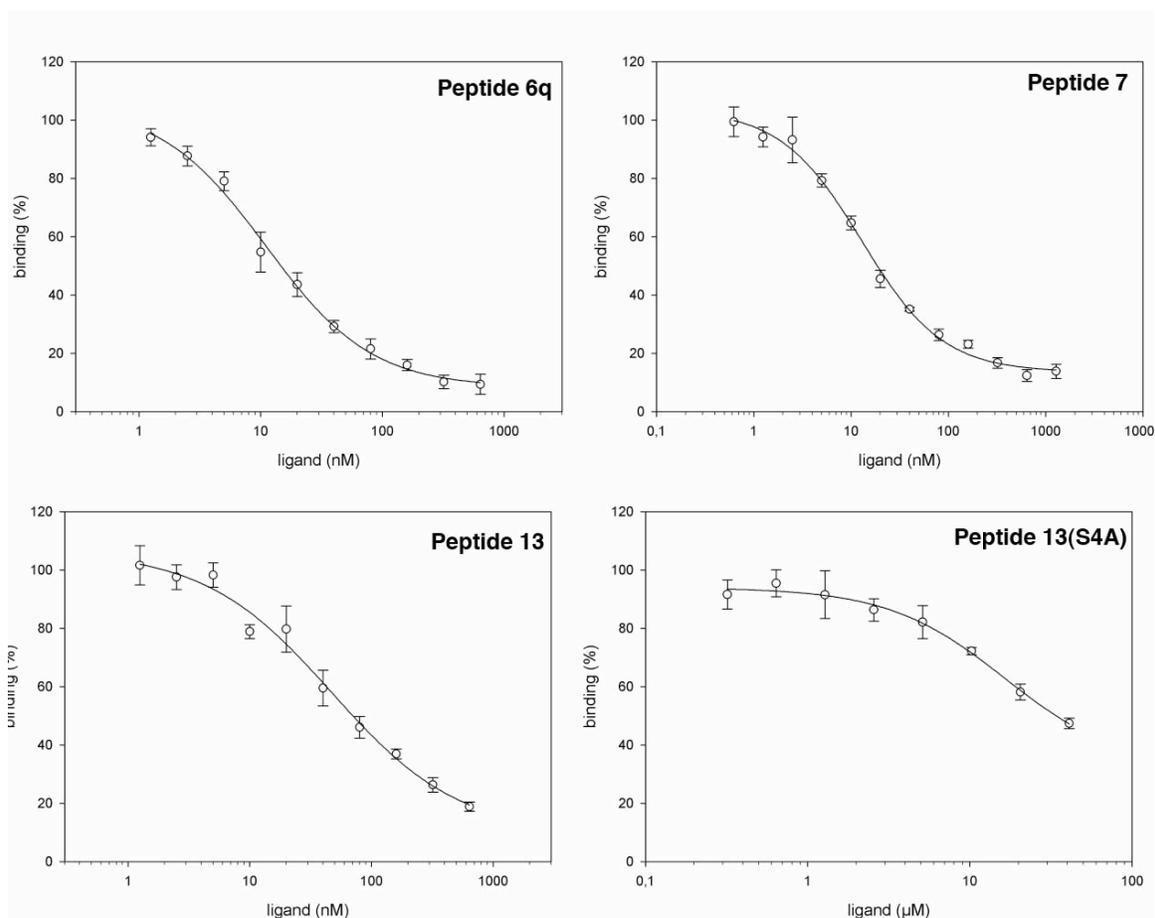


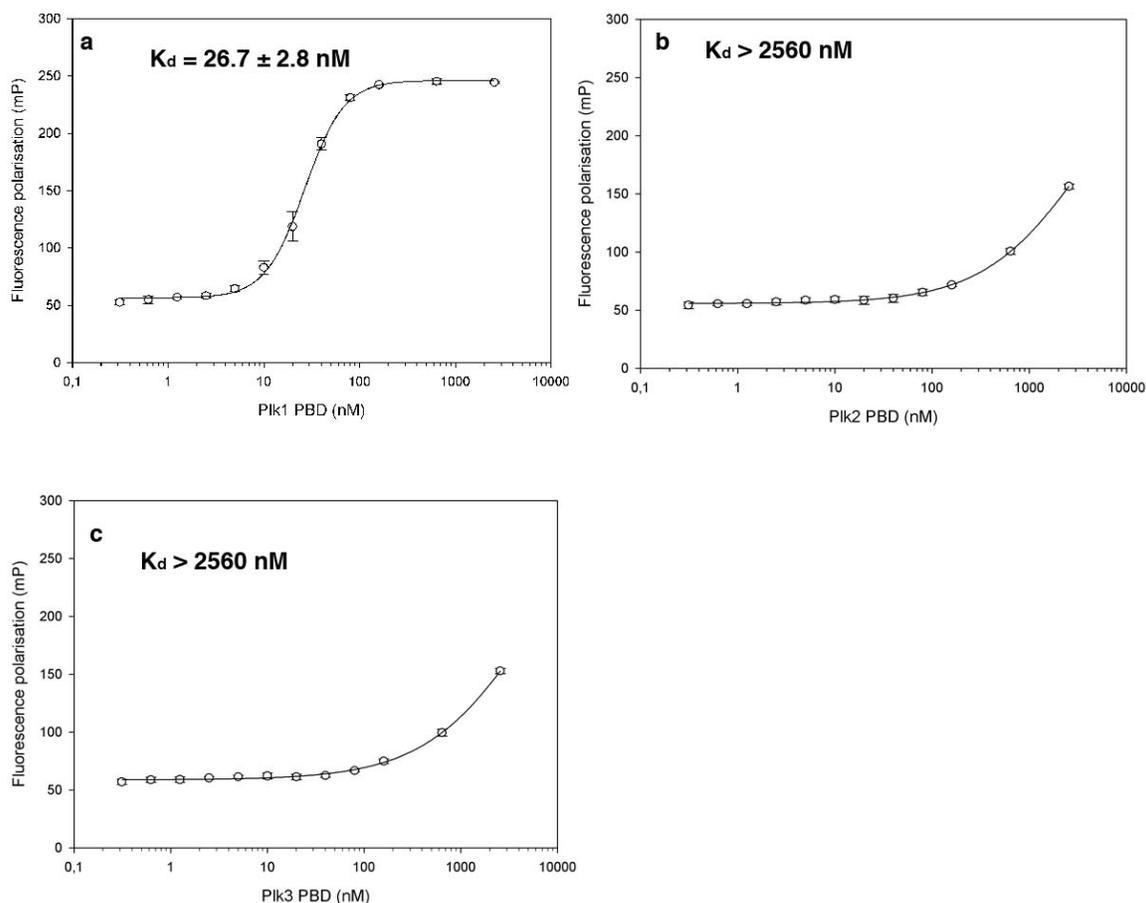
Figure S2. Results of fluorescence polarization assays showing inhibition of binding of 5-carboxyfluorescein-GPMQSpTPLNG-OH ((5-CF-15) and the Plk1 PBD as described in the Experimental Procedures of the published text with numerical IC_{50} values being shown in Table 1 of the published text.

PBD Fluorescence Polarization Binding Assays for Plk1, Plk2 and Plk3.

Binding assays for the PBDs of Plk1, Plk2, and Plk3 were performed essentially as described.^[4] In brief, fluorescein-labeled peptide **14** (final concentration: 2 nM) was incubated with the PBDs of Plk1, Plk2, and Plk3, respectively (final concentrations of buffer components: 50 mM NaCl, 10 mM Tris (pH 8.0), 1 mM EDTA, 0.1% Nonidet P-40 substitute, and 1 mM dithiothreitol). Proteins were used at final concentrations ranging from 0.039 nM to 2560 nM. Fluorescence polarization was analyzed 60 minutes after mixing of all components in a 384-well format using a Tecan Infinite F500 plate reader. Binding curves were fitted using SigmaPlot (SPSS). All experiments were performed in triplicate. K_d -values were extrapolated from the binding curves as the concentration at

which 50% of the fluorescein-labeled peptide is protein-bound. Dissociation constants (K_d) and binding curves for peptide **14** are shown in Figure S3.

Figure S3. Binding between fluorescein-labeled peptide **14** and the PBDs of (a)



Plk1; (b) Plk2, and (c) Plk3, respectively, as described above. Binding constants are shown for each domain.

X-ray Crystallography of Peptide **6q in Complex with Plk1 PBD Protein:**

Protein Purification and Crystallization. Plk1 PBD protein (residues 371-603) was purified as previously described.^[1] Crystals were grown using the hanging drop vapor diffusion method. PBD protein at 12 mg/mL in 10 mM Tris pH 8, 0.5 M NaCl, 10 mM DTT, 1% DMSO and 1 mM peptide **6q** was mixed with an equal volume of reservoir solution consisting of 36% (w/v) PEG 3350, 2% glycerol, 0.1 M HEPES pH 7 and 300 mM NaCl. Crystals began appearing overnight and reached maximum size over several days. Crystals grew in clusters that were manually broken up to obtain sufficiently single crystals suitable for data collection.

Data Collection, and Structure Determination and Refinement. Crystals were cryo-protected in reservoir solution supplemented with 1 mM of peptide **6q**, 1% DMSO and 10 mM DTT, and data were collected at 100 K on a Rigaku Raxis-IV image plate detector with a Rigaku RU-300 home X-ray source. The data were processed with the HKL^[2] and CCP4^[3] software suites. The structure was solved by molecular replacement using AMoRe^[4] using chain A of structure 3FVH^[1] (RCSB accession code) as a search model, and refined using PHENIX^[5] with manual fitting in XtalView.^[6] Refinement statistics are provided in the Supporting Information (Table S4) as well as the SigmaA weighted 2Fo-Fc electron density map around peptide **6q** (Figure S3).

Table S4. Data collection and refinement statistics for peptide 6q bound to Plk1 PBD.

PBD ID	XXXX
Space group	P2 ₁ 2 ₁ 2 ₁
a (Å)	57.6
b (Å)	58.6
c (Å)	66.9
Resolution range (Å)	20-1.55
Average redundancy	7.1
Completeness ¹	93.7% (56.6%)
R _{sym} ¹	0.076 (0.256)
Average I/σ ¹	17.5 (4.2)
R/R _{free} (%)	18.3 / 21.3

¹Values for the highest resolution shell are shown in parentheses.

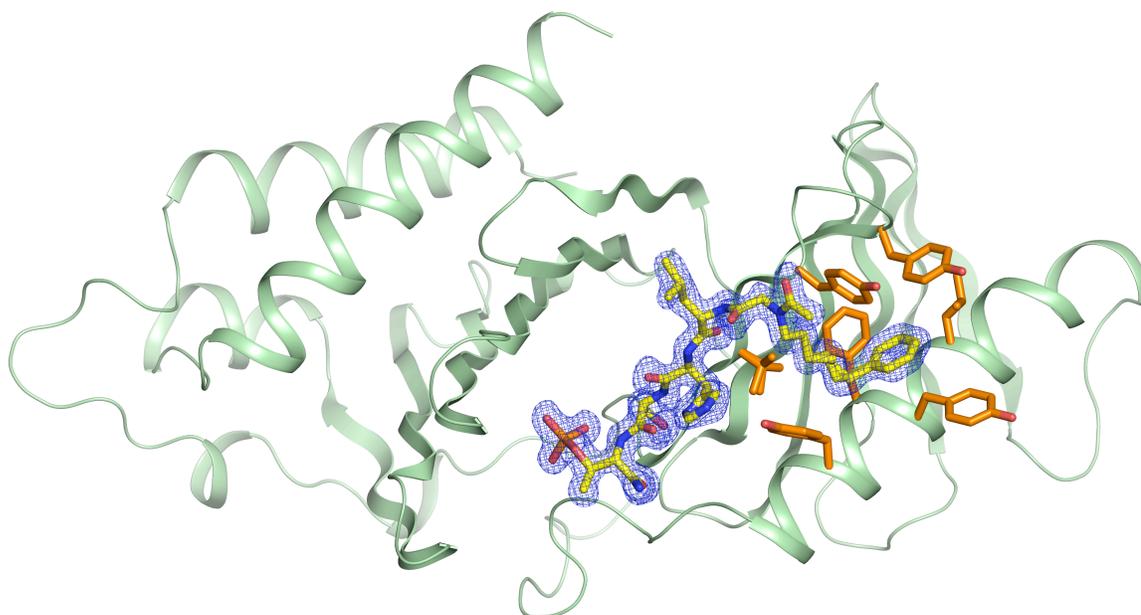


Figure S4. SigmaA weighted 2Fo-Fc electron density map contoured at 1σ and 1.55 \AA resolution around the ligand **6q** (stick rendering with yellow carbons). The PBD protein is shown in green cartoon rendering with hydrophobic side chains around the phenyl-alkyl substituent on **6q** shown in stick rendering with orange carbons. The V415 side chain and the phenyl-alkyl substituent of **6q** were modeled with alternate conformers.

References

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